Orally administered dipeptidyl peptidase-4 inhibitor (alogliptin) prevents abdominal aortic aneurysm formation through an antioxidant effect in rats

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Objective: **Dipeptidyl peptidase-4 (DPP-4) inhibitor, a novel antidiabetic drug, has a cardioprotective effect on ischemia-reperfusion injury through an antioxidant effect. However, the effect of DPP-4 inhibitor on aneurysm formation has not been investigated. We aimed to test the hypothesis that the DPP-4 inhibitor, alogliptin, attenuates vascular oxidative stress and thus inhibits abdominal aortic aneurysm (AAA) formation.**

Methods: **AAAs were created with intraluminal elastase and extraluminal calcium chloride in 36 male rats. Rats were divided into three groups: a low dose of alogliptin group (group LD; 1 mg/kg/d), a high-dose group (group HD; 3 mg/kg/d), and a control group (group C, water). Alogliptin was administered by gastric gavage once daily beginning 3 days before surgery. On day 7 after aneurysm preparation, reactive oxygen species (ROS) expression was semiquantified by dihydroethidium staining, and the oxidation product of DNA produced by ROS, 8-hydroxydeoxyguanosine (8-OHdG), was measured by immunohistochemical staining. Blood glucose concentrations were measured. Hematoxylin and eosin and elastica Van Gieson stainings were performed on day 28, and the AAA dilatation ratio was calculated.**

Results: **On day 7 (six in each group), dihydroethidium staining of the aneurysm wall showed a reduced level of ROS expression (4.6 ± 0.6 in group C, 2.7 ± 0.3 in group LD, and 1.7 ± 0.5 in group HD; $P < .0001$) and showed fewer 8-OHdG-positive cells in alogliptin-treated samples (138.1 ± 7.4 cells in group C, 102.5 ± 4.5 cells in group LD, and 66.1 ± 4.5 cells in group HD; $P < .0001$) The treatment significantly reduced messenger RNA expression of matrix metalloproteinases (MMPs) in aneurysm walls (relative expression: MMP-2: 2.1 ± 0.4 in group C, 1.3 ± 0.3 in group LD, and 0.9 ± 0.2 in group HD; $P < .001$; MMP-9: 2.0 ± 0.5 in group C, 0.3 ± 0.3 in group LD, and 0.3 ± 0.2 in group HD; $P < .001$). On day 28 (six in each group), the aortic wall in groups LD and HD was less dilated (dilatation ratio: 199.2% ± 11.8% in group C, 159.6% ± 2.8% in group LD, and 147.1% ± 1.9% in group HD; $P < .02$ group C vs HD) and had higher elastin content than in group C. The difference in blood glucose levels among the three groups was not significant.**

Conclusions: **The DPP-4 inhibitor, alogliptin, attenuates aneurysm formation and expansion dose-dependently in a rat AAA model via an antioxidative action. (J Vasc Surg 2014;59:1098-108.)**

Clinical Relevance: **Abdominal aortic aneurysm (AAA) is a potentially life-threatening condition of rupture, resulted in high mortality. Mechanisms of AAA formation have been under investigation and remain unclear. Because an effective pharmaceutical treatment for AAA has not been established, most patients are monitored by annual serial radiologic evaluation, without any treatment. Alogliptin is an antidiabetic drug that suppresses AAA formation without affecting normal glucose tolerance. Orally administered alogliptin has some clinical advantages in safety and convenience. Alogliptin could be an effective medical therapy for patients with a small AAA or patients in poor general condition who have been refused a surgical procedure.**

Abdominal aortic aneurysm (AAA) is a potentially fatal condition because of the risk of rupture. Ruptured AAA is the cause of 1% to 2% of all deaths worldwide.[1,2] The incidence of AAA has increased during the past 2 decades.[3] There is currently no effective prophylaxis for AAA except invasive surgical or endovascular therapies.

Reactive oxygen species (ROS) have been shown to play a role in many chronic diseases,[4-6] including cardiovascular diseases such as atherosclerosis.[7,8] and hypertension.[9,10] Enhanced production of ROS is associated with localized inflammatory responses and can cause progressive cell and tissue damage, which in the aorta eventually leads to AAA.[11] Although pharmacologic treatment for AAA has not been established clinically, experimental studies have demonstrated that genetic and pharmacologic inhibition of ROS production can inhibit aneurysm formation.[12,13] We recently reported that the free radical scavenger edaravone prevented aneurysm formation by ROS inhibition in the rat AAA model, which has proved that oxidative stress plays a crucial role in pathogenesis of AAA formation.[14]

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Dipeptidyl peptidase-4 (DPP-4, CD26) inhibitor, a novel antidiabetic drug, has been shown to exert its cardioprotective effect during ischemia-reperfusion injury by the reduction of ROS production.[15] However, the effect of DPP-4 inhibitor on aneurysm formation has never been investigated. In the present study, we tested the hypothesis that the DPP-4 inhibitor, alogliptin, prevents aneurysm formation through ROS inhibition in an experimental rat AAA model.

METHODS

Rats. The handling and use of laboratory animals in the experiments in this study conformed to the Guidelines for Animal Experimentation at Kobe University Graduate School of Medicine (Permission No. 100608) and the Guide for the Care and Use of Laboratory Animals (www.nap.edu/catalog/5140.html). The study used 36 male Sprague-Dawley rats (8 weeks of age, SLC Inc, Shizuoka, Japan).

AAA preparation. The experimental AAA model was created by means of simple application of intraluminal elastase and extraluminal calcium chloride, according to our recent report.[16] Briefly, rats anesthetized by intraperitoneal administration of pentobarbital (1 mg/kg) were placed under an operating microscope Leica M651 (Leica Microsystems, Heerburgg, Switzerland) at original magnification ×6 to ×10. After a midline abdominal incision and exposure of the abdominal aorta, 30 U elastase (135 U/mg; Elastin Products, Owensville, Mo) was applied to a 1-cm length of the infrarenal aorta intraluminally plus 0.5M $CaCl_2$ (Sigma-Aldrich, Tokyo, Japan) extraluminally for 20 minutes. Intraluminal saline infusion alone was performed to elucidate the barotrauma against the abdominal aortic wall as a preliminary experiment, which is discussed in the limitations. The abdominal incision was closed in layers, and the rats were allowed to recover.

CD26/DPP-4 in aneurysm wall. CD26, also known as DPP-4, is a multifunctional type II transmembrane glycoprotein[17] expressed in a variety of cells, including T cells, B cells, macrophages, inflammatory mononuclear cells, and vascular endothelial cells[18-21] and involved in T-cell activation.[22] Inhibition of DPP-4 signaling exerts antiatherosclerotic effects and reduces inflammation via inhibition of monocyte activation and chemotaxis.[23] On day 7, we assessed the expression of CD26-positive cells in the AAA wall by immunohistochemistry and evaluated messenger (m)RNA expression of CD26 by quantitative real-time polymerase chain reaction (RT-PCR) analysis.

Alogliptin administration. Alogliptin was provided by Takeda Pharmaceutical Company Ltd (Osaka, Japan). Two different doses (1 and 3 mg/kg/d) were tested for their effect on the prevention of AAA. Both doses were previously reported to be sufficient for inhibition of DPP-4 activity without decreasing the blood glucose level in normal glucose tolerance.[24] The 36 rats were randomly divided into three groups of 12 rats each: group LD received low-dose alogliptin (1 mg/kg/d), group HD received high-dose alogliptin (3 mg/kg/d), and group C received water as the control treatment. Alogliptin or water was given by gastric gavage once daily, starting 3 days before induction of the AAA.

Blood glucose levels. All rats were fasted overnight, and the blood glucose levels were measured with the Glutest E II system (Sanwa Chemical Lab, Nagoya, Aichi, Japan).

Macroscopic assessments. On day 28, animals were anesthetized, and the abdominal aorta was exposed. The aneurysm diameter, defined as the maximal dimension, and the aortic diameter at the infrarenal proximal neck site, were measured under physiologic conditions with an optical micrometer before harvest. The dilatation ratio (%) was calculated according to the following formula: Dilation ratio (%) = (maximal aneurysm diameter/normal aortic diameter) × 100. The harvested aortic aneurysm was divided into three portions. The first portion was used for pathologic examination, the second for in situ imaging of superoxide, and the third for quantitative RT-PCR analysis.

Histologic analysis. Paraffin-embedded 5-μm-thick sections were stained with hematoxylin and eosin (H&E) for general histologic analysis on day 7 and 28 and with elastica Van Gieson (EVG) for elastin on day 28. Images of the sections were captured with a Biozero microscopic system (BZ 8100, KEYENCE Co, Osaka, Japan) and assessed using Dynamic cell count BZ-HIC software (KEYENCE). Areas of elastin in a cross-sectional aortic wall were measured using Dynamic cell count BZ-HIC software. Elastin content was calculated by dividing the elastin-positive area by the cross-sectional media area of the aortic wall, which was measured using the EVG-stained sections to highlight internal elastic lamina and external elastic lamina, and expressed as a percentage. Microscopic digital images of five random fields on each section were scanned in a frame composed of a 500- × 380-μm rectangle.

Immunohistochemistry. Immunohistochemical staining was performed on paraffin-embedded sections on day 7 with monoclonal antibody specific for 8-hydroxydeoxyguanosine (8-OHdG; Japan Institute for the Control of Aging, Shizuoka, Japan), for CD26 (BD Biosciences, Erembodegem, Belgium, #559639) and for CD68 for macrophage (Abcam, Cambridge, Mass; #ab125212). Briefly, deparaffinized sections were incubated overnight at 4°C with primary antibody against 8-OHdG (5.0 μg/mL), CD26 (0.5 μg/mL), or CD68 (1 μg/mL). After three washes, sections were incubated for 30 minutes with secondary antibody antimouse immunoglobulin G (K4001; Dako, Japan) for 8-OHdG and CD26 and with secondary antibody antirabbit immunoglobulin G (K4003; Dako, Japan) for CD68 at room temperature. Microscopic digital images of five random fields on each section were scanned in a frame composed of a 500- × 380-μm rectangle, and the number of 8-OHdG-positive cells in aneurysm wall was counted using Dynamic cell count BZ-HIC software.

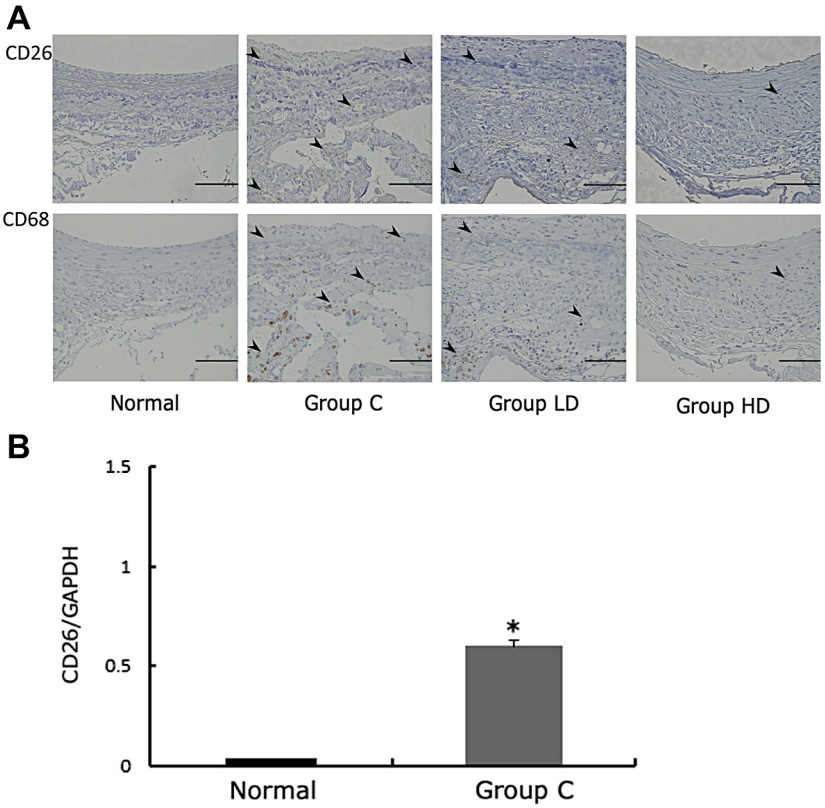

Fig 1. A, Immunohistochemical staining of aortic walls at day 7. Sections were stained with CD26 antibody (*upper panels*) and CD68 antibody (*lower panels*) in the control group (*group C*), and the low-dose (*LD*) and high-dose (*HD*) groups. The *arrowheads* indicate positive cells (*scale bar* = 100 μm). **B,** Messenger RNA expression of CD26 at day 7 in the normal native aorta (*normal*) and aneurysm wall in the control group that received water (*group C*). Statistical analysis was done using the Student *t*-test. $*P < .001$ vs normal. The *range bar* shows the standard deviation.

In situ imaging of superoxide. The harvested aortic aneurysm was embedded in Tissue-Tek O.C.T. Compound (Sakura Finetek, Zoeterwoude, Netherland), immediately frozen, and cut into 10-μm sections. Dihydroethidium (DHE; Invitrogen, Tokyo, Japan) was used to evaluate tissue superoxide levels in situ, as previously described.[25] Day 7 and 28 sections were incubated with DHE in phosphate-buffered saline in a dark, humidified chamber for 30 minutes at 37°C. DHE fluorescence was detected through a 580-nm filter, and DHE fluorescence images were scanned with a Biozero microscope in a frame composed of 500- × 380-μm rectangles. DHE fluorescence of arterial sections was quantified using Dynamic cell count BZ-HIC software. The mean fluorescence was semiquantified and expressed relative to values obtained for normal rat.

Quantitative RT-PCR analysis. Total RNA was isolated from the aneurysm samples using an RNeasy fibrous tissue mini-kit (Qiagen, Valencia, Calif) according to the manufacturer's instruction. The RNA was reverse-transcribed to complimentary DNA and amplified using a high-capacity complimentary DNA reverse-transcription real-time kit (Applied Biosystems, Foster City, Calif). Tissue from the day 7 aneurysm wall underwent PCR analysis for quantitation of mRNA expression of MMP-2, MMP-9, and CD26 using the ABI Prism 7500 sequence detector system (Applied Biosystems) with TaqMan universal PCR master mix and TaqMan real-time PCR primers (Applied Biosystems). The expression level of each mRNA was divided by mRNA expression level of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase.

Gelatin zymography. Proteins from aortic specimens on day 7 were extracted using a buffer containing 50mM Tris-HCl (pH 7.5), 150mM NaCl, 1% Triton X-100, 0.2% sodium dodecyl sulfate, and 1 mM ethylenediaminetetraacetic acid, supplemented with protease inhibitors (20 μg/mL aprotinin, 10 μg/mL leupeptin, and 1 mM phenylmethylsulfonyl fluoride). To determine gelatinolytic activities of MMP-2 and MMP-9 in the aorta, a gelatin-zymography kit (Primary Cell Co, Hokkaido, Japan) was used according to the manufacturer's instructions. The protein concentration was standardized with a microbicinchoninic acid protein assay kit (Pierce, Rockford, Ill). Aliquots of protein (20 μg) underwent sodium dodecyl sulfate gel electrophoresis, and densitometric

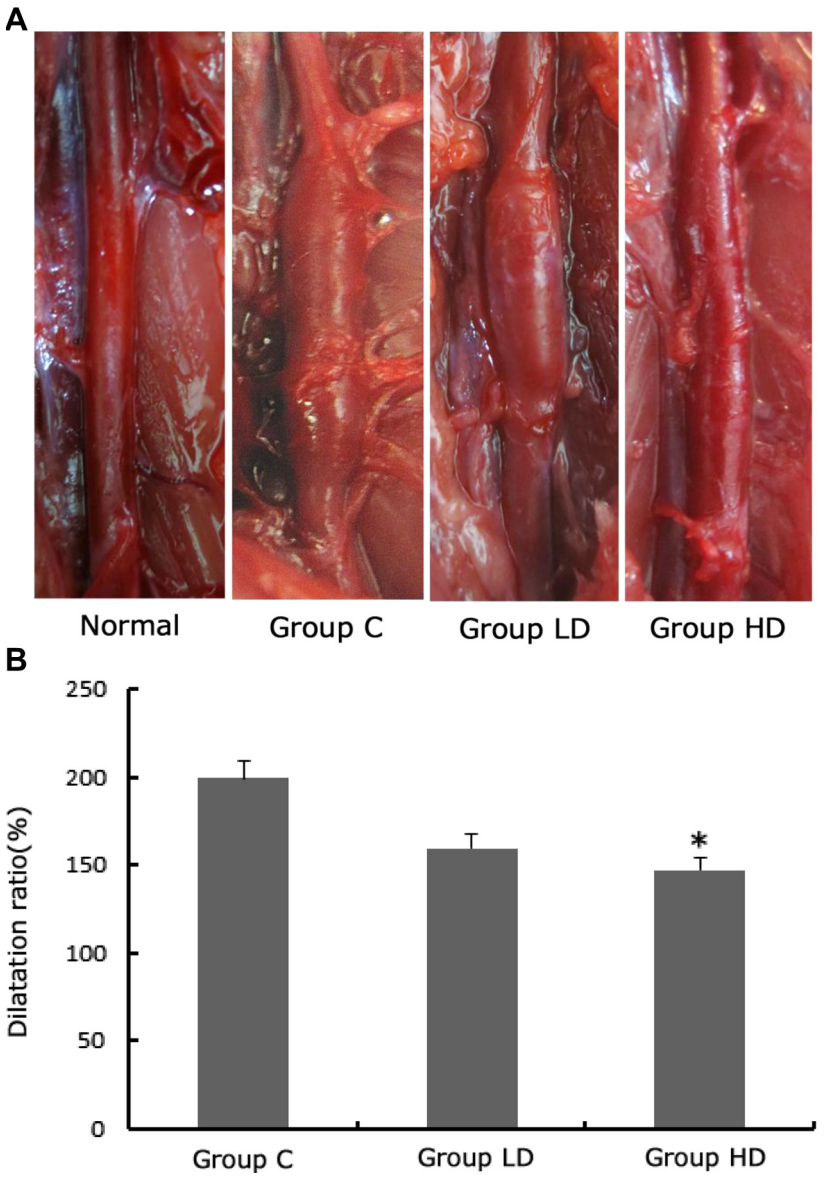

Fig 2. A, Aneurysm formation of abdominal aorta at 28 days in each group. Rats in the control group (*group C*) received water, rats in the low-dose group (*group LD)* received 1 mg/kg/d alogliptin, and rats in the high-dose group (*group HD*) received 3 mg/kg/d alogliptin. **B,** Dilatation ratio in each group. **P* < .02 group C vs HD. The *range bar* shows the standard deviation.

analysis of the lytic bands was performed by ImageJ software (National Institutes of Health, Bethesda, Md).

Statistical analysis. Continuous variables are expressed as mean ± standard deviation. Comparisons among multiple groups were performed using one-way analysis of variance, whereas comparisons between two groups were made using the Student *t*-test. All *P* values were two-sided and considered statistically significant at $P < .05$. If the one-way analysis of variance was significant, a post hoc analysis with the post hoc Games-Howell test was used to determine the significance among the groups. Data were analyzed with SPSS 17 software (SPSS Inc, Chicago, Ill).

RESULTS

The surgical procedure in each group required ~30 minutes to complete. Success rate was 100%. No technical failures or deaths occurred during the surgical procedures, and all animals survived to the end points. Six rats in each group were used for biochemical examinations of ROS expression, immunohistochemistry, RT-PCR analysis, and gelatin zymography on day 7. Another six rats in each

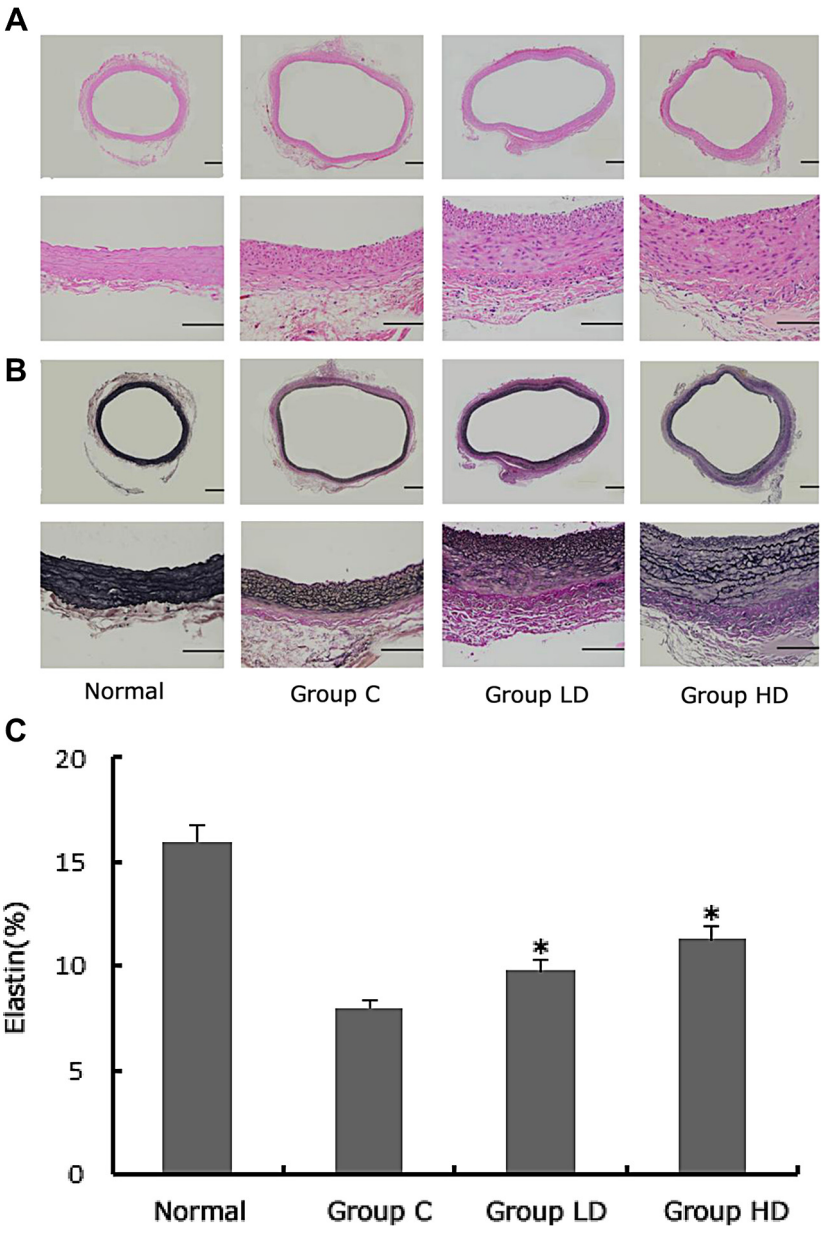

Fig 3. Histologic findings of abdominal aortic aneurysm at day 28. Sections were stained with **(A)** hematoxylin and eosin and **(B)** elastica von Gieson stain in each group (*upper panels*: original magnification, ×40; *scale bar* = 300 μm; *lower panels*: original magnification, ×400; *scale bar* = 100 μm). **C,** Quantitative analysis of elastin content is shown. Data are expressed as mean ± standard deviation (*range bars*) for six rats per group. *Group C,* Rats received water; *group HD,* rats received high-dose alogliptin (3 mg/kg/d); *group LD,* rats received low-dose alogliptin (1 mg/kg/d); *normal,* normal native aorta, Statistical analysis was by one-way analysis of variance with post hoc Games-Howell test. **P* < .0001 vs all other groups.

group were used for the measurement of aortic dilatation ratio and histologic examination (H&E, EVG staining) on day 28.

CD26/DPP-4 expression in aneurysm wall. The CD26 protein on the mononuclear cells was stained in AAA walls (Fig 1, *A*, *upper panel*) and mRNA expression of CD26 was significantly higher in AAA walls than in the aortas of control animals (Fig 1, *B*). Immunohistochemical staining of CD68 confirmed that CD26 protein was expressed on macrophages (Fig 1, *A, lower panel*).

Blood glucose levels. Blood glucose levels did not differ significantly among the groups and was 105.3 ± 3.6 mg/dL in group C, 105.5 ± 15.0 mg/dL in group LD, and 102.3 ± 6.1 mg/dL in group HD.

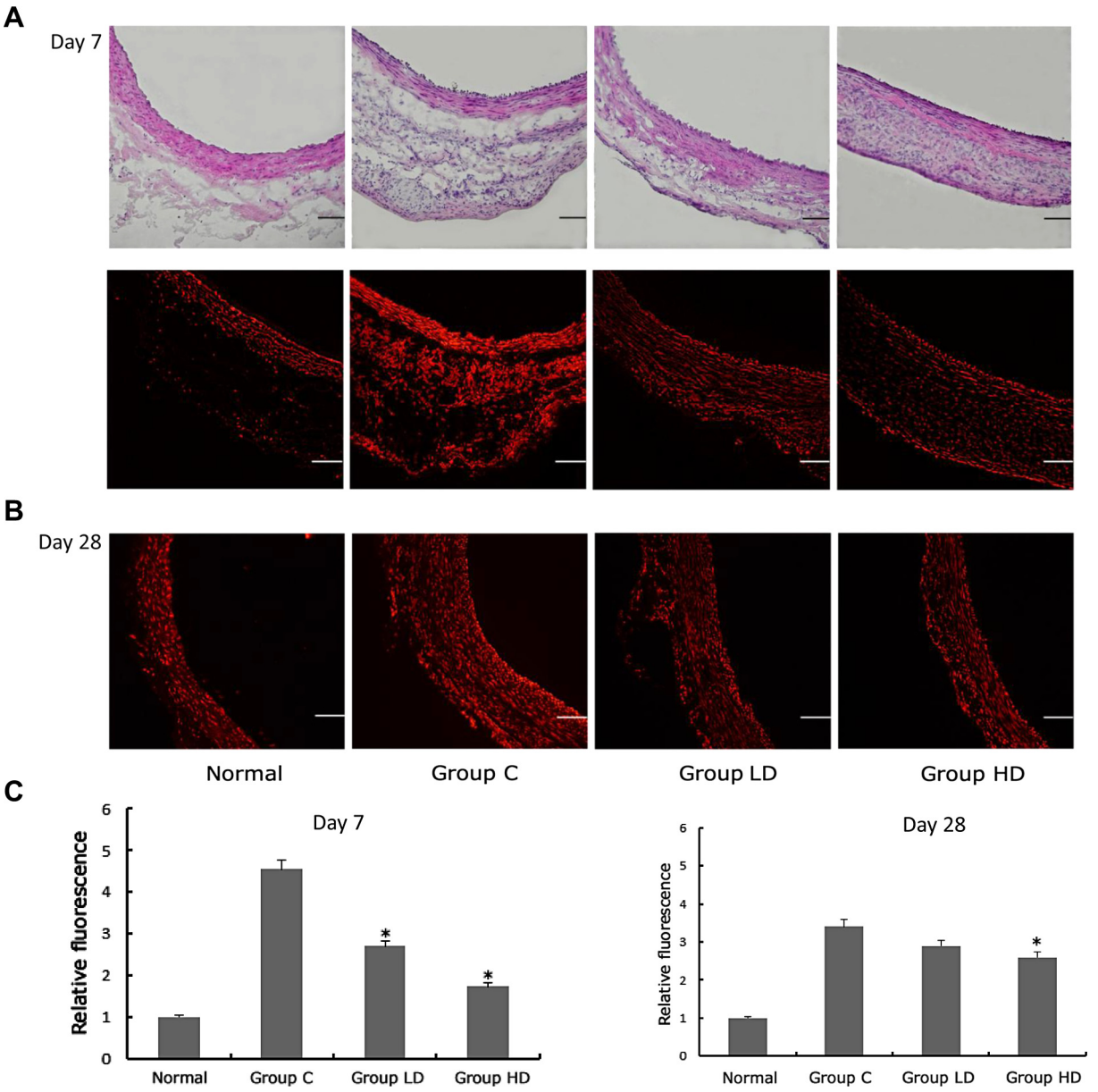

Fig 4. A, Reactive oxygen species expression stained with dihydroethidium in aneurysm walls at day 7 is shown in the *lower panels* (original magnification, ×400), whereas the *upper panels* show correspondent hematoxylin and eosin staining in each group. **B,** Reactive oxygen species expression in aneurysm walls at day 28 is shown (original magnification, ×400; *scale bar* = 100 μm). **C,** The mean fluorescence in a high-power field. All data are expressed as mean ± standard deviation (*range bars*) for six rats per group. Statistical analysis was by one-way analysis of variance with post hoc Games-Howell test. **P* < .01 vs all other groups on day 7, **P* < .01 group C vs HD on day 28. *Group C,* rats received water; *group HD,* rats received high-dose alogliptin (3 mg/kg/d); *group LD,* rats received low-dose alogliptin (1 mg/kg/d); *normal,* normal native aorta.

Macroscopic assessments. Macroscopic findings on day 28 are shown in Fig 2, *A*. Aortic dilatation and dilatation ratio in DPP-4-treated groups (groups LD and HD) were significantly lower than that in the water-treated group C (dilatation ratio: 199.2% ± 11.8% in group C, 159.6% ± 2.8% in group LD, and 147.1% ± 1.9% in group HD; $P < .02$ group C vs HD; Fig 2, *B*).

Microscopic assessments. Microscopic findings on day 28 are shown in Fig 3, *A* and *B*. The aortic wall in group C was the thinnest, with loose adventitia, whereas aortic walls in groups LD and HD were more thickened, accompanied with intimal hyperplasia and robust adventitia. Elastin was regenerated in the media in groups LD and HD. Elastin content in the aortic wall increased

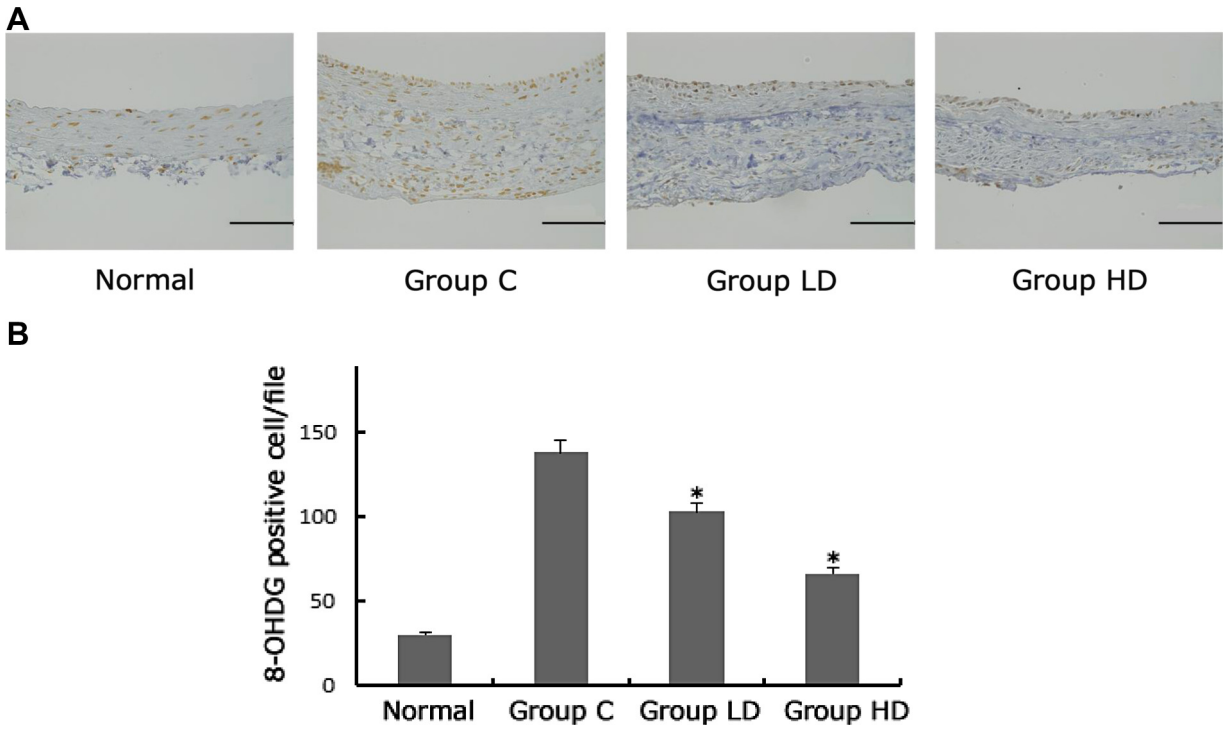

Fig 5. A, Immunostaining of 8-hydroxydeoxyguanosine (8-OHdG)-positive cells in aneurysm walls at day 7 is shown (original magnification, ×400; *scale bar* = 100 μm). **B,** The number of 8-OHdG cells was counted in a high-power field. All data are expressed as mean ± standard deviation (*range bars*) for six rats per group. Statistical analysis was by one-way analysis of variance with post hoc Games-Howell test. **P* < .0001 vs all other groups. *Group C,* rats received water; *group HD,* rats received high-dose alogliptin (3 mg/kg/d); *group LD,* rats received low-dose alogliptin (1 mg/kg/d); *normal,* normal native aorta.

significantly in DPP-4-treated groups compared with group C (8.0% ± 0.6% in group C, 9.8% ± 0.7% in group LD, and 11.3% ± 0.4% in group HD; $P < .0001$ vs all other groups; Fig 3, *C*). This increase in elastin was dose-dependent, being lowest in groups C and correspondingly higher in groups LD, and HD.

Effect of DPP-4 inhibitor on ROS expression and 8-OHdG in AAA walls of rats. To evaluate ROS expression in the aneurysm wall on days 7 and 28 (Fig 4, *A* and *B*), DHE staining was performed on sections of aorta from each group. In each group on day 7, the most intense DHE staining was basically observed on endothelial cells in the intima and smooth muscle cells in the media, which was confirmed by correspondent H&E staining in each group using serial sections. Another staining was observed in the loose tissue of the adventitia in group C. Similar findings were observed in the adventitia in groups LD and HD.

The DHE staining on day 7 showed that ROS expression in the aneurysm wall was significantly lower in the alogliptin-treated group compared with the control group (4.6 ± 0.6 in group C, 2.7 ± 0.3 in group LD, and 1.7 ± 0.5 in group HD; $P < .01$ vs all other groups), whereas a significant difference was observed between group C vs group HD on day 28 (3.4 ± 0.3 in group C, 2.9 ± 0.3 in the group LD, 2.6 ± 0.4 in group HD; $P < .01$ group C vs HD; Fig 4, *C*). The number of 8-OHdG-positive cells in aneurysm walls was significantly lower in alogliptin-treated groups than in the control group (138.1 ± 7.4 cells in group C ,103 ± 4.5 cells in group LD, and 66.1 ± 4.5 cells in group HD; $P < .01$ vs all other groups; Fig 5, *A* and *B*). A significant dose-dependent decrease in ROS expression and 8-OHDG staining was observed in the aneurysm walls between groups LD and HD.

Effect of DPP-4 inhibitor on MMPs expression and activity. mRNA expression of MMP-2 and MMP-9 was significantly lower in alogliptin-treated groups than in the control group (MMP-2: 2.1 ± 0.4 in group C, 1.3 ± 0.3 in group LD, 0.9 ± 0.2 in group HD; MMP-9: 2.0 ± 0.5 in group C, 0.3 ± 0.3 in group LD, 0.3 ± 0.2 in group HD; Fig 6, *A*). Gelatinolytic activities of MMP-2 and MMP-9 were also significantly lower in alogliptin-treated groups compared with the control group (MMP-2: 124.3 ± 5.1 in group C, 78.5 ± 6.6 in group LD, and 68.6 ± 16.8 in group HD; proMMP-2: 126.7 ± 10.7 in group C, 85.3± 3.4 in group LD, and 66.8 ± 17.7 in group LD; MMP-9:

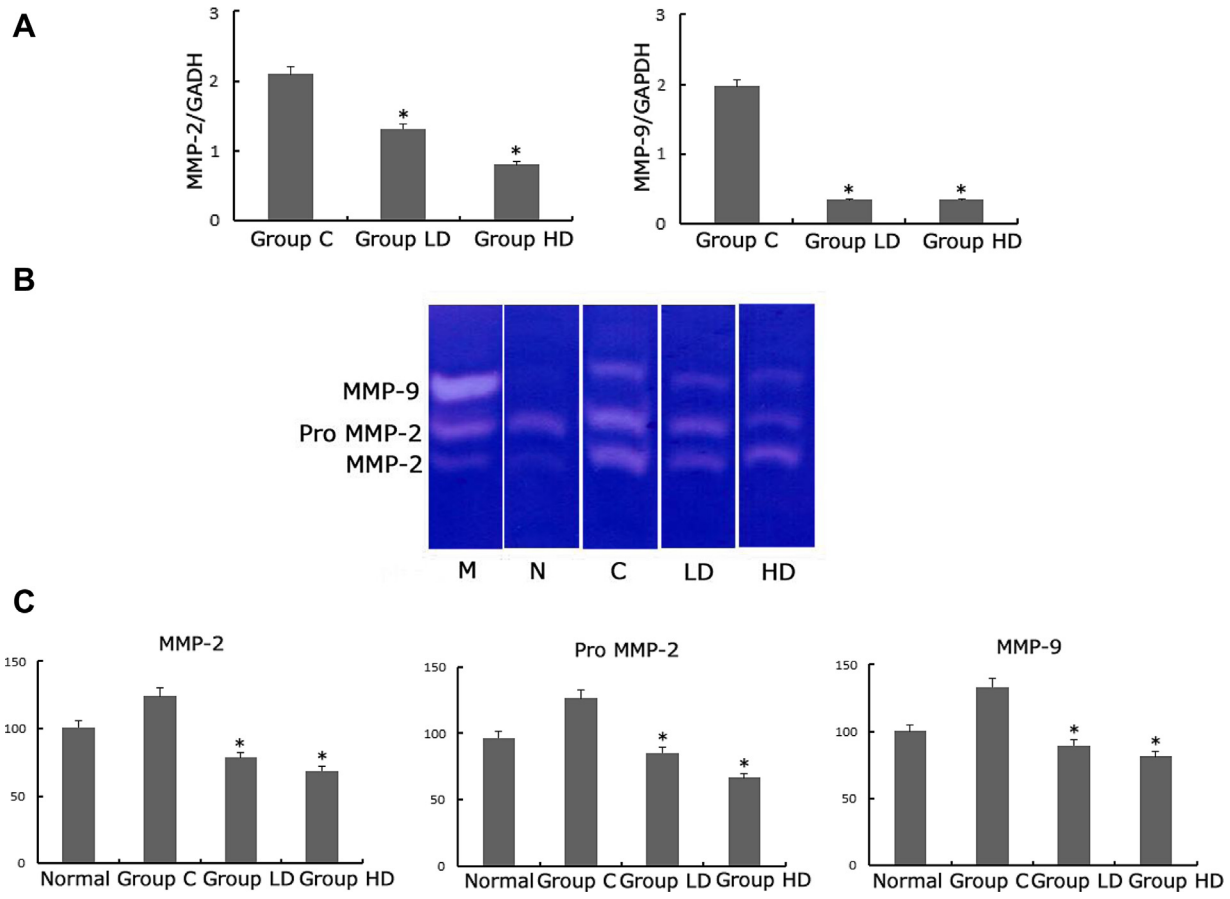

Fig 6. A, Messenger RNA expression of matrix metalloproteinase (*MMP*)-2 and MMP-9 in the aneurysm wall at day 7, with glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) as the housekeeping gene. **P* < .01 vs group C. **B,** Gelatin zymography demonstrates gelatinolytic activity of MMP-2 and MMP-9. **C,** Densitometric analysis of MMP-2 (*left*), Pro-MMP (*middle*) and MMP-9 (*right*) activities. All data are expressed as mean ± standard deviation for six rats per group. Statistical analysis was by one-way analysis of variance with post hoc Games-Howell test. **P* < .01 vs group C. *Group C,* rats received water as the control; *group HD,* rats received high-dose alogliptin (3 mg/kg/d); *group LD,* rats received low-dose alogliptin (1 mg/kg/d); *normal,* normal native aorta.

133.3 ± 17.2 in group C, 89.6 ± 8.5 in group LD, and 81.3 ± 10.1 in group HD; Fig 6, *B* and *C*).

DISCUSSION

This study showed that the DPP-4 inhibitor, alogliptin, can prevent AAA formation through an antioxidative action by a mechanism that is similar to its cardioprotective effect against ischemia-reperfusion injury by reduction of ROS production.[15] The results of the current study suggest that alogliptin might be an effective oral pharmacologic agent for AAA treatment in clinical practice.

ROS have been shown to play a central role in many chronic disease, including cardiovascular diseases such as atherosclerosis and hypertension.[5,9,26] Increased production of ROS is associated with localized inflammatory responses that can cause progressive cell and tissue damage, which has been reported to be closely linked to the pathogenesis of AAA.[11,27] Excessive production of ROS mediates activation of matrix-degrading enzymes and inflammation, resulting in the reduction of medial elastin content and aortic wall thinning, leading to aneurysm.[7,27-30] In this study, aneurysm formation was also associated with increased ROS expression and oxidative DNA damage (8-OHdG-positive cells) in the aortic wall. Expression of MMP-2 and MMP-9 mRNAs was increased in the aneurysm walls.

Aneurysm formation can be theoretically inhibited by antioxidants. We proved that ROS play a crucial role in aneurysm formation in this rat AAA model.[14] Other antioxidants, such as apocynin[13] and vitamin E,[31] have been recently reported to prevent AAA formation. DPP-4 inhibitor is widely used for patients with type 2 diabetes mellitus. As mentioned above, DPP-4 inhibitor suppressed expression of ROS in an animal model of ischemia-reperfusion injury.[15] The present study demonstrated that the DPP-4 inhibitor, alogliptin, has antioxidant properties that can

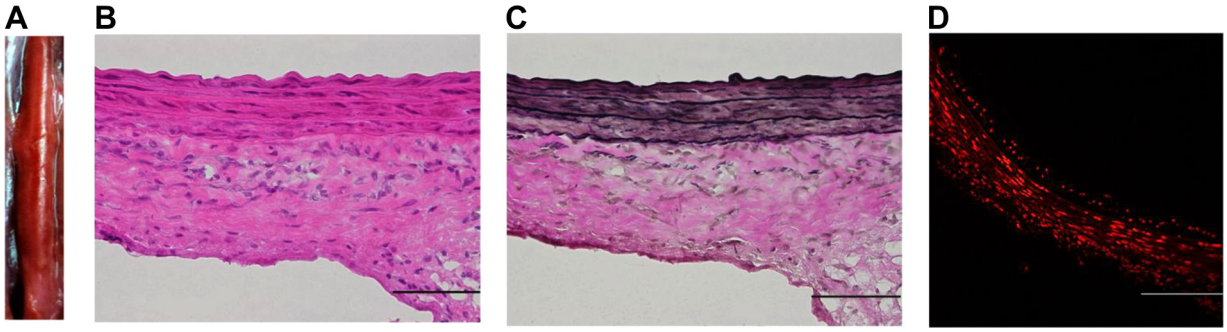

Fig 7. A, Aneurysm formation in the abdominal aorta at 7 days in the saline group. **B** and **C,** Histologic findings of abdominal aorta at day 7 in the saline group. Sections were stained with hematoxylin and eosin (H&E; **B**) and elastica von Gieson (EVG; **C**) (original magnification, ×400). **D,** Reactive oxygen species expression is shown in aortic walls at day 7 in the saline group (original magnification, ×400).

act as a powerful ROS scavenger in the process of AAA formation. Alogliptin suppressed not only ROS dose-dependently, particularly in the early phase (day 7), but also recruitment of inflammatory CD68/CD26-positive cells, which prevented the destruction of medial elastin in the current AAA model. MMP-2 and MMP-9 mRNA expression was reduced, and the activities of the enzymes in the aortic wall were effectively decreased through ROS elimination by alogliptin treatment. Notably, even treatment with a low-dose of alogliptin decreased the mRNA expression of MMP-9 as effectively as a high-dose treatment. Previous studies have shown that MMP-2 and MMP-9 are required for AAA formation[32,33] and that ROS play a major role in the activation of MMPs.[29] These findings strongly suggest that alogliptin ameliorates aortic wall degeneration by reducing oxidative stress, resulting in the prevention of aortic dilatation.

A novel finding in the present study is that orally administered alogliptin can prevent aneurysm formation. Oral administration has many clinical advantages and applicability. Effective pharmacotherapy for AAA might be of significant benefit for patients with small AAA or those in poor general condition who cannot undergo surgical procedures. Some pharmacologic agents have been reported to prevent AAA formation but have not been used clinically. We recently reported the efficacy of edaravone against AAA.[14] However, edaravone is clinically used by intravenous administration. We believe that orally administered alogliptin has a high potential as a clinical therapeutic agent for AAA without affecting normal glucose tolerance.

Alogliptin has some clinical side effects, such as headache and dizziness, with doses >400 mg,[34] but no side effects were noted with the 3 mg/kg/d in this study with rats or in a previous report.[24] The side effects of several pharmaceutical agents with inhibitory effect on AAA formation have been reported.[35,36] Statins have side effects, such as acute kidney failure and liver failure,[37] and angiotensin-receptor blockers also have side effects such as hypotension[38] and kidney failure.[39] Further studies to test other doses of this DPP-4 inhibitor are required to evaluate the balance between its efficacy and safety in the prevention of AAA formation.

This study has some limitations that deserve attention. This AAA model is simple and easy to perform and is highly reliable and reproducible to create a saccular aneurysm.[16] When we used normal saline intraluminally instead of elastase, aortic dilatation was 126.3% on day 7 (Fig 7, *A*), which did not meet the criteria of aneurysm in the current study. Indeed, the surgical procedure has a small amount of barotrauma against the aortic wall. H&E and EVG staining in the group demonstrated a little thicker aortic wall than that of normal aorta; however, elastin content stained by EVG seemed to be preserved on day 7 (Fig 7, *B* and *C*). DHE staining in the group (Fig 7, *D*) demonstrated increased expression of ROS compared with normal aorta, but this was weaker than that in control aorta (group C, elastase and calcium chloride). However, the stress was similarly applied to the aortic walls in all groups.

Because this AAA model in rats is created by elastase and calcium chloride, it does not completely mimic the human AAA because it lacks several prominent features of the human lesion such as atherosclerosis and intraluminal thrombosis.

The third limitation is the lack of evaluation of proinflammatory cytokines and the analysis of signal transduction for antioxidative effects by alogliptin. We proved the potential causal relationship between ROS inhibition and alogliptin's vascular protective effect indirectly by dose-dependent fashion.

CONCLUSIONS

This study demonstrated that the DPP-4 inhibitor, alogliptin, attenuates aneurysm formation dose-dependently in rat model of AAA via an antioxidative action and might be an effective oral pharmacologic agent for AAA treatment in clinical practice.

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AUTHOR CONTRIBUTIONS

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Final approval of the article: YO, KO
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